Modeling β-Lactam Interactions in Aqueous Solution through Combined Quantum Mechanics–Molecular Mechanics Methods

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ABSTRACT: In this article, we have carried out a series of theoretical computations intended to analyze the interactions of β-lactam compounds in aqueous solution. The final aim is to rationalize the influence of the medium on β-lactam antibiotics reactivity. In particular, the hydrolysis reaction has been studied because of the considerable interest due to its relationship with resistance mechanisms developed by bacteria. The study is extended to the simplest β-lactam molecule, propiolactam or 2-azetidinone, and to the corresponding hydroxylated complex (resulting from the addition of a hydroxyl anion to the carbonyl group) that plays a crucial role in hydrolysis processes. Molecular Dynamics simulations have been carried out using a hybrid quantum mechanics–molecular mechanics potential: the solute is described using the density functional theory, whereas water solvent molecules are treated classically. This represents a sophisticated computational level which, compared to usual force-field simulations, has the advantage of allowing a detailed analysis of solute's electronic properties. The discussion of results is focused on the role played by solute–solvent hydrogen bonds and solvent fluctuations on solute's structure. © 1999 John Wiley & Sons, Inc. J Comput Chem 20: 1401–1411, 1999

Keywords: β-lactams; solvent effects; quantum mechanics–molecular mechanics; molecular dynamics; density functional theory

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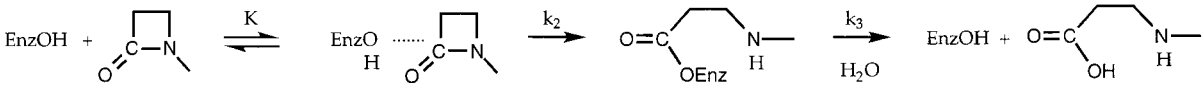
Introduction

Since Fleming observed the antibacterial action of penicillin at the end of the 1920s,[1] a large family of antibiotics with a common feature, the possession of a β-lactam ring, has been developed. These substances present a vast spectrum of action and a low toxicity that converts them in the antibacterial agents most widely employed. The β-lactam antibiotics execute their bactericidal action by interrupting the function of a group of enzymes (penicillin binding proteins, PBPs) that catalyze the synthesis of the peptidoglycan strands of the bacterial cell wall.[2] It was proposed that the antibiotic is a structural analogue of the D-alanyl-D-alanine peptide fragment, and the enzyme mistakes it for its substrate.[3] Unfortunately, bacteria have developed different ways of resistance, the most important being the hydrolytic action of β-lactamase enzymes.[4] From the previous statements, one understands the large interest raised by the study of these compounds and their mechanism of action. This research has been tackled from different perspectives. Earliest works were focused on the relation between the strain of the four-membered ring or the reduced amide resonance with the antibiotic activity, although evidence to support these ideas was ambiguous.[5] Nowadays, the availability of crystal structures for some PBPs[6] and β-lactamases,[7–14] together with kinetic and mutagenesis studies,[15] has led to an improved understanding of the chemistry and mode of action of β-lactam antibiotics.

Most of the β-lactamases (classes A and C), and PBPs are active-site serine enzymes. In Scheme 1, we represent the accepted overall mechanism for the enzyme + antibiotic reaction.[4] In the first stage, the antibiotic and the enzyme form a noncovalent enzyme–substrate complex. Then, the hydroxyl group of the serine residue of the active site is acylated by the β-lactam carbonyl group, and finally the acyl-enzyme is hydrolyzed, regenerating the enzyme and producing the degradation of the antibiotic. If k_3 is large, the enzyme is a β-lactamase. Conversely, if k_3 is small, the enzyme is inhibited. Despite this qualitative description, the elementary reaction steps remain a matter of controversy, especially in the area that concerns the proton transfer steps in the formation of the acyl–enzyme complex, i.e., the deprotonation of the serine hydroxyl group and protonation of the β-lactam nitrogen, for which several mechanisms have been proposed.[16–18]

Quantum mechanical calculations are useful to discern among potential reaction mechanisms, especially to get information at the molecular level. In a recent work, Wladkowski et al.[19] have used an *ab initio* approach to study a specific mechanism for the initial acylation step of the catalyzed hydrolysis, and have estimated the energetic effect of the oxy-anion hole components existing in these enzymes. On the other hand, the study of alcoholysis, and alkaline hydrolysis pathways of simple β-lactams has been also a subject of great interest,[20–23] because common features are expected for such reactions and enzymatic hydrolysis of antibiotic compounds.[24,25] In this sense, we have recently analyzed the mechanisms of both alkaline and neutral hydrolysis of *N*-methylazetidinone.[23] In these studies, the effect of the environment has been considered by means of a dielectric continuum model, and has been shown to influence the relative stability of the species involved in the reaction as well as the reaction mechanism itself. Apart from the effect due to long-range electrostatic interactions, specific solute–water interactions and dynamic solvent effects have been claimed to play an important role.[23,26] Because these aspects are difficult to study with simple continuum solvent approaches, further investigation is necessary, using more sophisticated models.

Thus, we study here the structure and dynamics of simple β-lactam compounds in aqueous solution using Molecular Dynamics simulations and hybrid quantum mechanics–molecular mechanics (QM/MM) potentials. In particular, the solvent molecules are described by a classical MM force field, whereas the solute is treated quantum mechanically using the Density Functional Theory (this potential will be referred to as DF/MM). The use of such an approach allows the accurate evaluation of time fluctuations of solute's structural



SCHEME I

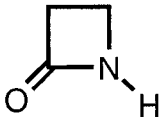
parameters and electronic properties, such as bond orders or net atomic charges. As we show below, these electronic and nuclear changes induced by the solvent may be determinant for the β-lactam ring reactivity. The results are compared to those obtained through the dielectric continuum approach.

The species considered here are the simplest β-lactam compound 2-azetidinone or propiolactam (**1**) and the corresponding hydroxylated complex (**2**). The latter has been included in the present study because it is a pertinent model of the tetrahedral intermediate, or acylated complex, formed prior to ring opening during the hydrolysis reaction. Indeed, the microscopic aspects of the acylation process are not clear, because the ring opening and nucleophilic attack of serine on the carbonyl may be a one-step or a two-step process.-According to recent *ab initio* calculations,[19,23] structure **2** is a reaction intermediate, at least in some of the possible hydrolysis reaction mechanisms, as shown in Scheme 2. The first step seems to be the rate-limiting one in the acyl–enzyme formation process.[19,23]

Methodology

CONTINUUM SOLVENT MODEL

Electrostatic solvent effects using a simple polarizable dielectric continuum model of the solvent have been computed using an ellipsoidal cavity shape and a multipolar expansion of the solvent electrostatic potential (up to sixth order in this work). The method is well documented in the literature,[27,28] and is not further described here. It should simply be pointed out that the approach allows to account explicitly for polarization effects on both the solute's electronic and nuclear charge distribution. The dielectric constant of the continuum was that of liquid water (78.4).



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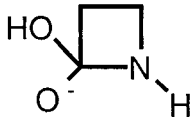
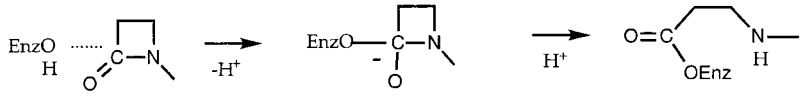


2

DF/MM MOLECULAR DYNAMICS IN AQUEOUS SOLUTION

In our combined DF/MM MD study, the classical subsystem is composed by 300 water molecules, and the quantum subsystem is either propiolactam (hereafter β-lactam) or the corresponding hydroxylated compound. These have been selected because they are the simplest model for β-lactam antibiotics. For the quantum part, we used the VWN[29] local exchange-correlation function with a double-zeta quality basis sets including polarization functions on nitrogen, carbon, and oxygen atoms with contractions: H(41), N(621/41/1), O(621/41/1), and C(621/41/1) (hereafter DZP basis). The auxiliary basis sets for the electron density and exchange-correlation fit were (4; 4) for hydrogen and (4, 3; 4, 3) for the rest of atoms (see program deMon[30] for details). Lennard–Jones parameters for quantum atoms were taken from ref. 31, and the TIP3P potential[32] was selected for the classical water molecules. Details on the computation of the coupled QM/MM term are given elsewhere.[33] Analytical forces are obtained from the derivatives of the energy with respect to the positions of quantum nuclei and classical sites. Simulations have been carried out at the NVT ensemble in a cubic box of 20.9 Å of side at 25°C using the Nosé–Hoover algorithm.[34] Periodic boundary conditions and a cutoff distance of 10.4 Å have been applied. Quantum hydrogen atoms have the mass of deuterium.

Equilibration of the aqueous solution of the neutral β-lactam or its hydroxylated form was started from configurations obtained through classical simulation runs.[26]Additional equilibration was carried out in both cases during 20,000 steps using a time step of 1.5 fs. The geometry of the quantum molecules was kept fixed using the RATTLE algorithm,[35] and was taken from geometry



Tetrahedral Intermediate

SCHEME II

optimizations at the same quantum level (VWN/DZP) in which the interaction with the solvent was incorporated by means of a continuum model.[27,28] After equilibration, a rigid-body simulation of 30.0 ps with a time step of 1.5 fs was run to obtain well-defined radial distribution functions. Then, starting from the last point of these simulations, 10,000 steps of unconstrained Molecular Dynamics were run for both systems with a time step of 0.75 fs.

Results

AVERAGED ELECTROSTATIC SOLVENT EFFECTS

First of all, solvent effects have been estimated by means of the polarizable continuum model because these results can be useful as reference values. Selected values of the geometry optimizations in gas phase and aqueous solution at the (VWN/DZP) level are compiled in Table I. In the β-lactam molecule, the CN bond has a noticeable double bond character, as reflected by the short bond length (1.373 Å) and the value of the Mayer's bond order (1.253 a.u.). This bond is strengthened when passing from the gas phase to solution, diminishing the bond length (1.362 Å) and increasing the bond order (1.324 a.u.). This is accompanied by a simultaneous weakening of the double CO bond, which is lengthened from 1.215 Å in the gas phase to 1.232 Å in solution. The same features can be observed in the case of the hydroxylated complex, when passing from the gas phase to solution the CN bond is shorthened (from 1.546 to 1.535) while the CO bond is lengthened (from 1.276 to 1.299 Å). On the other hand, when comparing the hydroxylated and the neutral β-lactam molecules, the effect of the acylation process on the β-lactam can be analyzed. The addition of the hydroxyl anion on the carbonyl carbon atom weakens both the CO and CN bonds, with a considerable increase of the CN bond length ($\Delta d = 0.173$ Å both in the gas phase and solution). This change is also clearly reflected in the CN and CO bond orders, which diminish when the hydroxyl group links the carbon atom. It is interesting to note that upon hydroxylation, the excess negative charge is partly transferred not only to the carbonyl oxygen atom (the net Mulliken charge increases by 0.217 a.u. in the gas phase and 0.223 a.u. in solution), but also to the nitrogen atom (0.068 a.u. in the gas phase and 0.135 a.u. in solution). Another important difference between the neutral and the hydroxylated forms is that the ring of the former remains planar (both in the gas phase and in solution), whereas in the later, the ring is distorted, having a ring dihedral angle (NCCC) of 20.2 degrees in the gas phase and 12.1 degrees in solution (the positive value indicates that the nitrogen atom is displaced in the opposite direction to the hydroxyl anion addition).

The effect of the solvent on the geometrical and electronic structure of the neutral β-lactam molecule and the hydroxylated complex, as models of β-lactam antibiotic and tetrahedral intermediate, respectively, can be rationalized in terms of valence bond language by assuming two main solute configurations, as shown in Figure 1. For the neutral molecule, the solvent interaction, which generally favors charge separation, will preferentially stabilize the right-hand zwitterionic structure of the upper part of Figure 1. This may explain the strengthening of the CN bond and the weakening

TABLE I.

Bond Lengths (Å), Ring Dihedral Angle (Degrees), Mayer's Bond Orders, and Mulliken Charges (a.u.) for Structures 1 and 2 at the VWN/DZP Level in the Gas Phase and in a Continuum Model.

	Neutral β-Lactam		Hydroxylated β-Lactam	
	$\varepsilon = 1.0$	$\varepsilon = 78.4$	$\varepsilon = 1.0$	$\varepsilon = 78.4$
d_{CN}	1.373	1.362	1.546	1.535
d_{CO}	1.215	1.232	1.276	1.299
Φ	0.0	0.0	20.19	12.12
B_{CN}	1.253	1.324	0.944	1.007
B_{CO}	2.124	2.037	1.913	1.852
Q_C	0.173	0.157	0.095	0.088
Q_N	−0.349	−0.353	−0.417	−0.488
Q_O	−0.279	−0.380	−0.496	−0.603

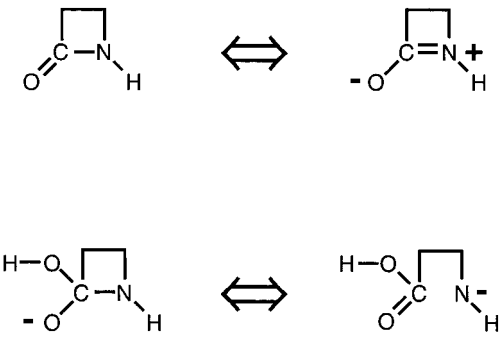


FIGURE 1. Valence bond structures proposed for the neutral and hydroxylated β-lactam molecules.

of the CO bond with solvation. In the case of the hydroxylated molecule (bottom part of Fig. 1) we can also consider two valence bond structures: one in which the negative charge is localized on the oxygen atom, with CO and CN simple bonds, and another one in which the negative charge is localized on the nitrogen atom, with a double CO bond and no bond between the carbon and nitrogen atoms. In solution, because of the larger accessibility of the oxygen atom to solvent molecules, the structure in which the negative charge is localized on this atom is more stabilized than the other one, resulting in a shortening of the CN bond with respect to the gas phase result. This strengthening of the CN bond would also explain the larger ring dihedral angle of the hydroxylated form in the gas phase with respect to the solution. Comparing the valence bond structures of the hydroxylated β-lactam with those of the neutral β-lactam, one can also explain the lengthening of the CN bond upon hydroxylation. In the structures proposed for the neutral species, double or single bonds exist between carbon and nitrogen atoms in contrast with the hydroxylated species where single or no bond is present. This qualitative picture, taken with some caution,[36] has already been employed to analyze amides reactivity.[37]

DYNAMICS IN AQUEOUS SOLUTION: RIGID SOLUTE

Radial distribution functions (RDFs) for selected quantum atoms of the β-lactam molecule (carbon plus oxygen atoms on carbonyl group and nitrogen atom) have been recorded along constrained DF/MM simulations in water. Using constrained geometries for the quantum subsystem we can employ a longer time step and, consequently, we can extend our simulation to longer times. The geometries of the quantum subsystems were taken from the continuum calculations presented above. This was a natural choice for equilibrating the system based on previous experiences with other systems.[38] As shown below, the geometric descriptions obtained from the continuum model and the DF/MM unconstrained simulation are not very different. RDF results are shown in Figures 2 and 3 for both neutral and hydroxylated β-lactam molecules. For the neutral molecule we can observe a well-structured solvation shell around the oxygen atom, the first peak of the hydrogen distribution function appearing at 1.75 Å. Integration of this first peak gives a coordination number for the carbonyl oxygen atom of about 2.8. In contrast

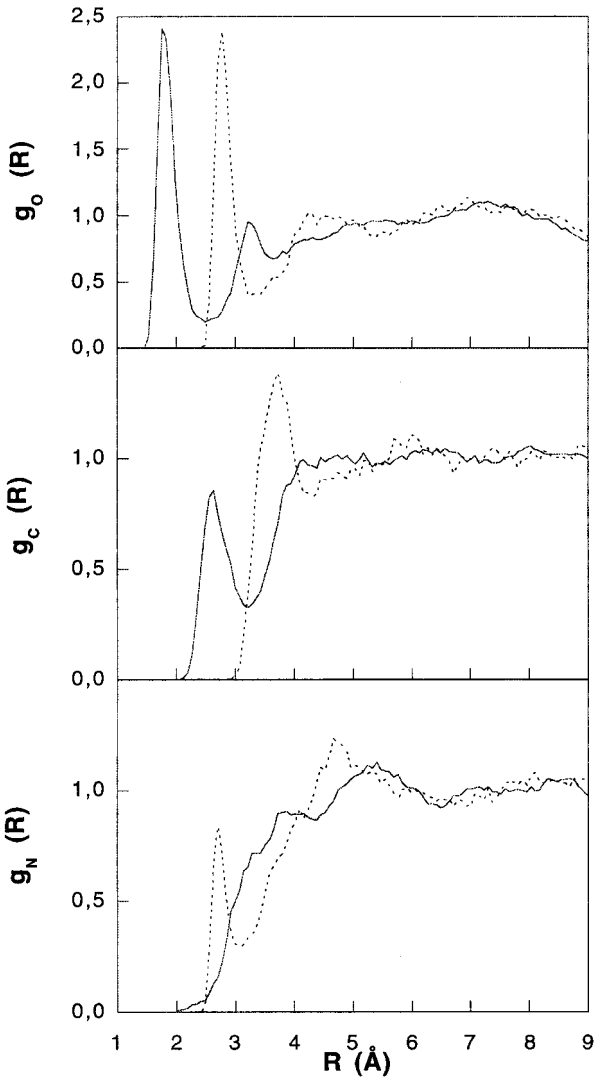


FIGURE 2. Radial distribution functions around carbonyl oxygen, carbonyl carbon and nitrogen atoms of the neutral β-lactam. The solid line corresponds to the water hydrogen, and the dashed line to the water oxygen.

with this result, the RDFs around the nitrogen atom indicates the absence of a well-defined solvation shell around it. The small peak of the water oxygen distribution function around the nitrogen atom is most probably due to the presence of solvent molecules hydrogen bonded to the hydrogen atom of the amide group. However, this fact is not very relevant for biologically active β-lactams because this amide hydrogen atom is substituted by other groups. More interesting is the fact that, in average, the nitrogen atom does not accept any hydrogen bond from water molecules. Similar results have been found by Gao et al. in a QM/MM simulation of formamide[39] and by us in a classical

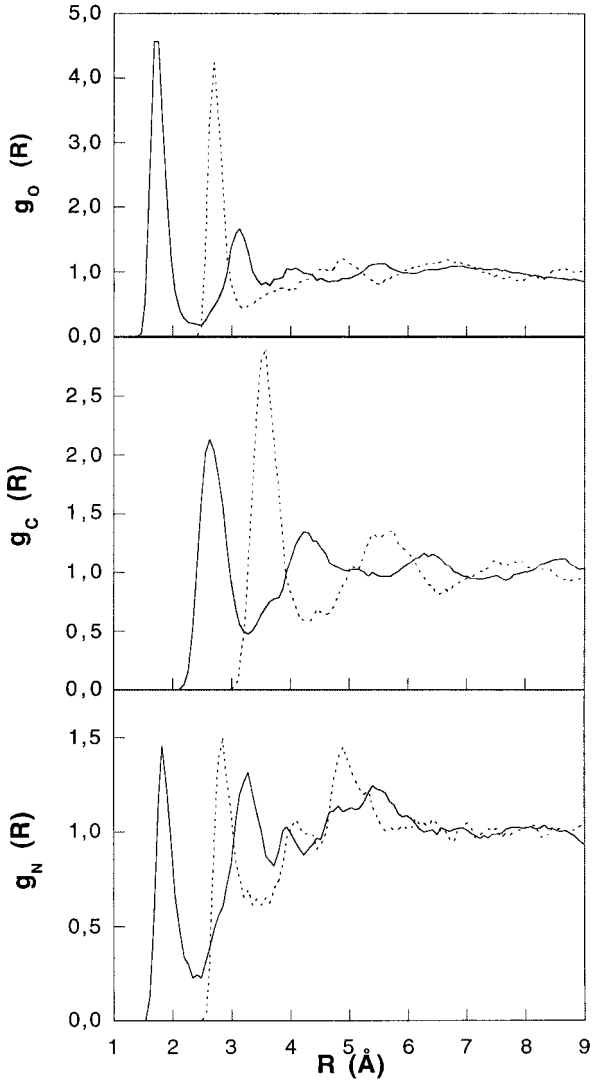


FIGURE 3. Radial distribution functions around carbonyl oxygen, carbonyl carbon, and nitrogen atoms of the hydroxylated β-lactam. The solid line corresponds to the water hydrogen, and the dashed line to the water oxygen.

Molecular Dynamics simulation of the *N*-methylazetidinone derivative.[26] It is interesting to note that previous studies on β-lactam–water complexes[23] also show the trend of water molecules to solvate the carbonyl oxygen preferentially.

The hydroxylated and neutral molecules present a similar structure for the RDFs around carbon and oxygen quantum atoms. However, as a consequence of the enhancement of the solute–solvent electrostatic interactions, due to the excess negative charge, the height of the first peaks are considerably increased in the hydroxylated molecule (note the different scales in Figs. 2 and 3). The variation is notable for the O atom because it carries a large part of the negative charge. The first peak, that corresponds to the H··O hydrogen bond, appears now at 1.68 Å and leads to an integrated coordination number of 4.1. Nevertheless, the main difference when comparing the RDFs of neutral and hydroxylated β-lactam molecules appears for the nitrogen atom. The RDFs now show a clear peak located at 1.82 Å for the solvent hydrogen atom and 2.85 Å for the solvent oxygen atom. Integration of the hydrogen RDF first peak gives a coordination number of 1.6. These results confirm the expected ability of the nitrogen atom of the tetrahedral intermediate to form hydrogen bonds with solvent molecules as opposed to the neutral β-lactam molecule.

Acylation has been proposed to be the first step of β-lactam hydrolysis (see Scheme 1). The computed differences between RDFs of the neutral and hydroxylated molecules suggest that hydroxylation, or more generally acylation, can be favored by the formation of hydrogen bonds with the carbonyl oxygen atom. The presence in some enzymes of a structure able to form such hydrogen bonds is known as the oxy-anion hole, and occurs not only in β-lactamases but also in other enzymes.[40] A second factor favoring acylation is the stabilization of the tetrahedral intermediate, simulated here by means of the hydroxylated form, with hydrogen bonds with the nitrogen atom. This may be important in enzymatic reactions because the oxyanion hole does not necessarily favor the cleavage of the CN bond in the global hydrolysis process. Indeed, *ab initio* calculations on the β-lactam enzymatic hydrolysis seem to show that the oxy-anion hole does not have a determinant effect on the rate-limiting energy barrier of the acylation process, which is found in the formation of the tetrahedral intermediate.[19]

DYNAMICS IN AQUEOUS SOLUTION: FLEXIBLE SOLUTE

Starting from the final configurations of the constrained MD trajectories, we started a 10,000 steps run (7.5 ps) both for the β-lactam molecule and the hydroxylated product with unconstrained geometries. The averaged values obtained for the CN and CO bond lengths, the NCCC ring dihedral angle, and some electronic properties of the systems are given in Table II. The trends observed with the continuum model are reproduced. However, an important enhancement of the solvent

TABLE II.
Averaged Values and Standard Deviations of Selected Bond Lengths (Å), Ring Dihedral Angle (Degrees), Mayer's Bond Orders, and Mulliken Charges (a.u.) for Structures 1 and 2 Obtained during 7.5 ps of Unconstrained MD DF/MM Simulation.

	Neutral β-Lactam		Hydroxylated β-Lactam	
	Averaged Value	s	Averaged Value	s
d_{CN}	1.353	0.044	1.516	0.048
d_{CO}	1.251	0.045	1.327	0.049
Φ	−0.46	5.61	13.87	5.92
B_{CN}	1.387	0.055	0.990	0.043
B_{CO}	1.914	0.068	1.678	0.076
Q_C	0.180	0.036	0.126	0.029
Q_N	−0.384	0.060	−0.569	0.037
Q_O	−0.490	0.058	−0.747	0.045

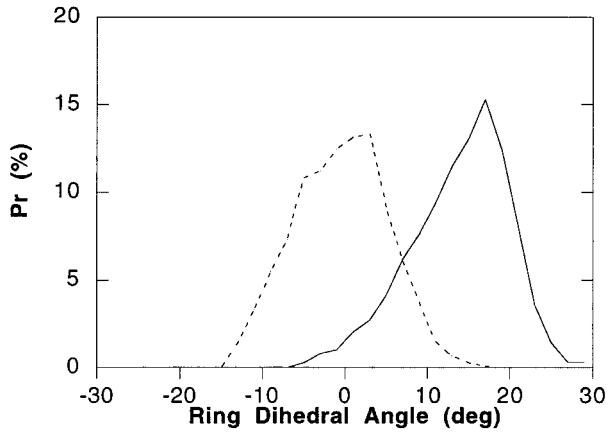


FIGURE 4. Probability distribution functions of the ring dihedral angle of the neutral (dashed line) and the hydroxylated (solid line) β-lactam molecules obtained after the 7.5 ps unconstrained MD simulation.

effects can be observed with respect to the continuum model calculations (see Table I). Strengthening of the CN bond and weakening of the CO bond with solvation are larger now than with the continuum model both in the neutral and hydroxylated molecules. The charge transfer towards the oxygen and nitrogen atoms of the β-lactam after hydroxylation is also enhanced (net Mulliken charges are increased by 0.257 and 0.185 a.u., respectively). It is also interesting to note here that releasing the internal geometry of the solute has a moderate effect on the average electronic properties of the system. So, for example, the Mulliken charges of the nitrogen and oxygen atoms of the neutral β-lactam molecule obtained in the rigid solute simulation are −0.371 and −0.458 a.u., respectively, very close to those obtained in the present flexible solute simulation. As discussed below, the average bond orders obtained from the constrained and unconstrained simulations are also quite similar.

The probability distribution function of the ring dihedral angles of both neutral and hydroxylated β-lactams are shown in Figure 4. Quite large oscillations are frequently observed for the ring dihedral angle of the neutral and hydroxylated β-lactam molecules, as reflected in the standard deviation values appearing in Table II (about 6 degrees in both cases). The distribution function for the neutral β-lactam molecule presents its maximum value around 0 degrees, with an averaged ring dihedral angle of −0.5 degrees. Thus, the β-lactam ring of the neutral molecule is planar both in the gas phase and in solution, although we have recently shown in a similar β-lactam molecule (*N*-methyl-2-azetidinone) and using a continuum model[26] that the deformation of this ring is easier in the gas phase than in solution. This is due to the increased double bond character of the CN bond after solvation. By means of cluster calculations with a small number of water molecules it is possible to obtain a minimum energy structure with solvent molecules hydrogen bonded to the nitrogen atom. In this structure, the neutral β-lactam is no longer planar and presents a ring dihedral angle of 8.2 degrees[23b] in the case of *N*-methyl-2-azetidinone, but we have not found evidence for such a minimum energy structure during our simulation. In fact, these nonplanar structures are most probably a consequence of the reduced number of solvent molecules used in the cluster calculations and, thus, they are not representative of the solution.[26] The present simulation seems to confirm this hypothesis.

In principle, several conformations are possible for the hydroxylated molecule, depending on the value of the ring dihedral angle.[23a] However, in our simulation, no conformational changes are observed, and the ring dihedral angle oscillates around an averaged value of 13.9 degrees. It is interesting to note the excellent agreement between the predicted ring dihedral angles using the continuum model and the MD averaged values, which are, in both cases, considerably lower that the gas phase dihedral angle. Thus, in accordance with our previous considerations about the solvent

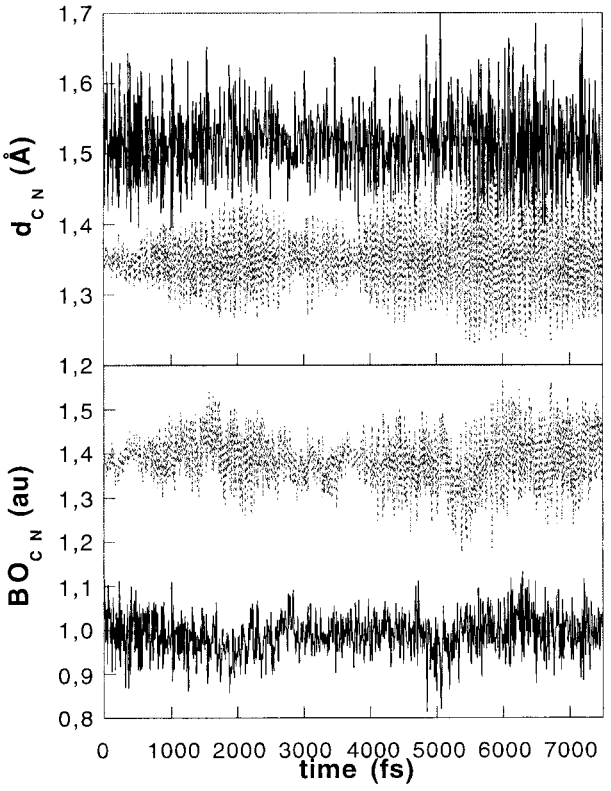


FIGURE 5. Evolution of the CN bond length (top) and the corresponding Mayer's bond order (bottom) for the neutral (dotted line) and the hydroxylated (solid line) β-lactam molecules during the 7.5 ps unconstrained MD simulation.

effects on β-lactam structures, deformation of the β-lactam ring of the hydroxylated species is also easier in the gas phase than in solution.

With the aim of studying the ring stability in solution, we have monitored the behavior of the CN bond along the simulation for the neutral and the hydroxylated β-lactam molecule. The properties of this bond (from the geometric and electronic points of view) are shown in Figure 5 along 7.5 ps. Oscillations with amplitudes of about ± 0.1 Å and ± 0.15 a.u. are observed for bond lengths and bond orders, respectively. Larger oscillations are observed in the hydroxylated molecule than in the neutral one, which is in agreement with the magnitudes of the CN bond orders in both molecules.

ANALYSIS OF POLARIZATION EFFECTS

We now analyze in some detail the solvent polarization effect on the electronic distribution of the β-lactam. Within this scope, it is useful again consider the valence bond structures in Figure 1. As pointed out above, the solvation effect tends to weaken the CO bond and strengthen the CN bond for the two systems considered. For this reason, the difference between CO and CN bond orders, $\Delta B = B(CO) - B(CN)$, appears to be a suitable electronic quantity for probing the relative weight of the formal valence bond structures as a function of the molecular environment. Computed ΔB values in different conditions are analyzed in Table III. Independent of the solvent model employed, the values of ΔB always decrease compared to gas phase quantities, although the change is notably larger when the discrete model is employed. Note that geometry relaxation (compares first and second rows) leads to a decrease of ΔB for the neutral molecule, as expected, but displays an increase for the hydroxylated system. The latter result is a little surprising, but is explained by the fact that in gas, the CO bond order of the hydroxylated system increases slightly by increasing the distance of the carbonyl carbon atom to the hydroxyl oxygen atom. The larger effect of the electronic polarization may be evidenced by comparing row 2 with rows 3 or 4. Finally, in the MD simulations, the results obtained using either the fixed geometry corresponding to the continuum model (row 4) and those obtained in the unconstrained simulation (row 5) are not quite different.

The preceding results give an idea of the importance of the average solvent effect. However, solvent dynamics may also play a role on the instantaneous characteristics of the bonds that could differ substantially from their mean values if fluc-

TABLE III.
Values of ΔB (i.e., difference in CO and CN Bond Orders, See Text) in Different Conditions for the Neutral and Hydroxylated β-Lactam Compounds Studied Here.

Environment	Geometry	Neutral β-Lactam	Hydroxylated β-Lactam
Gas Phase			
	gas[a]	0.871	0.969
	continuum[b]	0.838	0.985
Aqueous Solution			
Continuum	continuum[b]	0.713	0.845
MD simulation	constrained[b]	0.521	0.638
MD simulation	unconstrained	0.527	0.688

[a] Optimized geometry for the isolated system.
[b] Optimized geometry in solution using the continuum model.

tuations of the environment were large. Fluctuations that decrease either the number or the strength of water hydrogen bonds with the carbonyl oxygen should destabilize the zwitterionic formal structure of the neutral system. Similarly, for the hydroxylated β-lactam molecule, fluctuations involving desolvation of carbonyl oxygen should favor the right-hand valence bond structure that formally presents no CN bond.

To analyze the correlation between solvent fluctuations and solute bond orders, one must define a convenient global solvent coordinate that represents the polarization of the medium. The difference of solvent electrostatic potential on nitrogen and oxygen atoms, $\Delta V = V(N) - V(O)$ appears to be a good candidate. Note that this quantity has negative values because carbonyl oxygen atom is better solvated by hydrogen bonds than the nitrogen atom. So, solvent fluctuations leading to less negative ΔV are expected to favor nitrogen atom solvation and/or disfavor oxygen atom solvation. To separate the effects of solvent and solute fluctuations, we now use the results of the constrained simulation because in that case the time evolution of the solute's electronic polarization is only related to solvent dynamics.

The results obtained for ΔV and ΔB during 1 ps are shown in Figure 6 for the neutral and the hydroxylated β-lactam molecules. It is clear that ΔB and ΔV exhibit parallel evolutions during the simulation: when ΔV decreases in absolute value, the CO and CN bond orders change so that ΔB decreases also (note that because we are assuming the Born–Oppenheimer approximation, the electronic response of the solute to solvent changes is assumed to be instantaneous). The instantaneous ΔBs may differ by 20% (or more) with respect to their mean values. Although these changes represent a smaller variation than that predicted in going from gas to solution, they are significant and may have important consequences on the chemical reactivity of the species. In agreement with our valence bond picture, an increase in the number or in the strength of the hydrogen bonds with the oxygen carbonyl atom reinforces the β-lactam CN bond, while the opposite effect is obtained when the hydrogen bonds are established with the nitrogen atom. Thus, hydrogen bonds formed with the carbonyl oxygen atom both in the solution or in an enzymatic environment (the oxy-anion hole) may have two different effects on the hydrolysis process: they can assist the process stabilizing the charge developed on this oxygen atom, but also, as far as these hydrogen bonds strengthen the CN bond, they may render the deformation and breaking of the β-lactam ring more difficult.

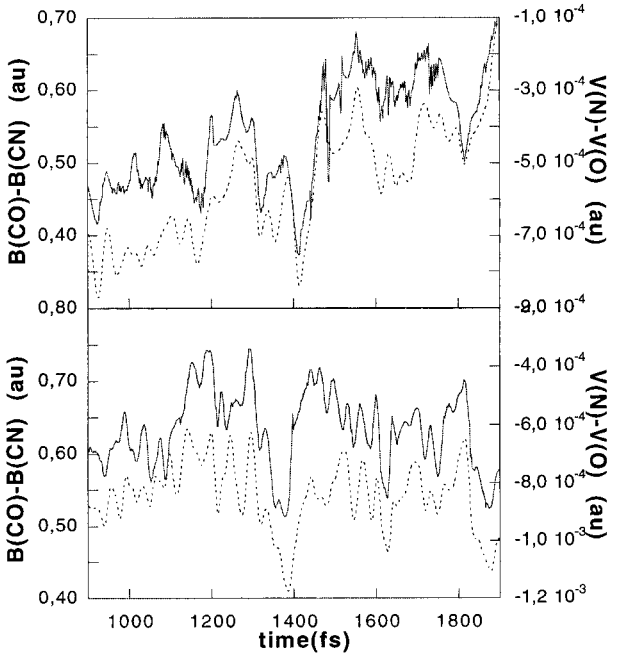


FIGURE 6. Differences between CO and CN bond orders (solid line) and between the solvent electrostatic potential measured on nitrogen and carbonyl oxygen atoms (dashed line) during 1 ps of a constrained MD simulation for the neutral (top) and hydroxylated (bottom) β-lactam molecules.

The aqueous solution simulations can be useful as a reference for analyzing the interactions of β-lactams with a β-lactamase enzyme. We can compare the structure of a proposed Michaelis complex of a β-lactam antibiotic with a class A β-lactamase[18] with the results of our study. In our simulation, the first peak of the solvent hydrogen-β-lactam oxygen radial distribution gives a coordination number close to 3, while in the proposed Michaelis complex only two hydrogen bonds are formed between the enzyme and the carbonyl oxygen atom. The so-called oxy-anion hole is formed by the backbone amide components of Ala237 and Ser70. Thus, the hydrogen bond interaction between the oxy-anion hole and the carbonyl oxygen is not as strong as the corresponding interactions in water solution. Furthermore, no structural order is predicted around the neutral β-lactam nitrogen atom in solution, while in the enzyme, the β-lactam nitrogen atom can form a hydrogen bond with the hydroxyl hydrogen of Ser130 at different steps of the hydrolysis process. In particular, such N··H interactions may be enhanced after acylation of the

carbonyl, as found in aqueous solution. Through these *N*-interactions, the enzyme may favor the acylation step with respect to solution energetics. Because this is done without unfavoring the CN breaking step, the enzyme–substrate interactions may lead to catalysis of the reaction. Some evidence on the active role of the Ser-130 residue has been observed after superimposition of the structures of the TEM-1 native enzyme and the acyl–enzyme complex with 6α-(hydroxymethyl) penicillanate. The side chain of Ser-130 is displaced after complexation and acylation, and the hydroxyl oxygen is found at 3.1 Å from the nitrogen atom of the acylated substrate.[41] This fact would be consistent with the previously described enhancement of hydrogen bonds of the β-lactam nitrogen atom after the nucleophilic attack. Moreover, site-directed mutagenesis of Ser-130 by asparagine, alanine, and glycine shows none or reduced enzymatic activity.[42] Considering the previous analysis of solvent fluctuations, these differences between β-lactam interactions in aqueous solution and in an enzymatic enviroment are expected to weaken the β-lactam CN bond in the enzyme with respect to the solution.

Conclusions

The combined DF/MM Molecular Dynamics simulation presented in this work represents the most sophisticated study reported to date for the solvation of β-lactam compounds in water. The main trends confirm the results obtained with simpler methods, like the dielectric continuum approach, as for instance, the variation of the equilibrium bond lengths through the effect of the medium. Nevertheless, the simulation also allows a detailed description of the solvation shells, and in this sense we have shown that the neutral β-lactam molecule presents substantial differences with respect to the hydroxylated complex. Analysis of the radial distribution functions show that the carbonyl oxygen atom in propiolactam is able to accept hydrogen bonds from the solvent but not the amide nitrogen. However, for the hydroxylated compound, both atoms exhibit a well-defined solvation shell. Fluctuations of the solvent water molecules around the solute produce important changes in the bond orders that may favor activation of the solute towards nucleophilic agents. These results are easily rationalized, assuming two main valence bond structures for the neutral and the hydroxylated β-lactam molecule. The displacement of the equilibria between these valence bond structures (see Fig. 1) can be used to predict the effect of a particular solvent fluctuation on the electronic structure of the solute.

Although aqueous simulations cannot mimic enzymatic environments, analysis of the interactions between the β-lactam and water molecules can be useful as a reference for the interactions found between the substrate and the enzyme. In this sense, comparison of the hydrogen bonding pattern of the β-lactam carbonyl oxygen and nitrogen atoms in solution and in the enzymatic environment shows significative differences that may explain some of the catalytic properties of the enzyme.

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